I hereby certify that this correspondence is being deposited with the United States Postal Service on the date set forth below as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date of Signature and Deposit: 12/22/04

Jean C. Baker
Attorney of Record

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Mary Lynne Perille-Collins, et al.
Serial No.:	10/692,889
Filed:	October 24, 2003
For:	HOST/VECTOR SYSTEM FOR EXPRESSION OF MEMBRANE PROTEINS
Group Art Unit:	1653
Examiner:	R. Mondesi

Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MARY LYNNE PERILLE-COLLINS

Dear Sir:

I, Mary Lynne Perille-Collins, declare that:

1. I am a named inventor in the above-identified case. I am in my 25th year as a faculty member at the University of Wisconsin-Milwaukee and have been a professor in the Department of Biological Sciences since 1993. My general field of study and expertise is microbial molecular biology. I have attached my curriculum vitae as Exhibit A.

2. Attorney Jean C. Baker has asked me to review the current Office Action in the above-identified case and comment on the Examiner's cited references. I disagree with the Examiner's characterization of the Hessner, et al., 1991 as either teaching or making obvious the present invention. I am the corresponding author of Hessner, et al., 1991.

3. The Examiner's rejection based on the publications of Hessner, et al. is due to misinterpretation of this work. In Hessner, et al., mutants in *puf* genes were constructed and complemented by DNA fragments. However, an expression vector was not used. The DNA fragments for complementation were delivered by a mobilizable cloning vector – pRK404E1. The vector has cloning sites in the lacZ gene. This facilitates cloning because the cloned fragment interrupts lacZ and the resulting recombinant clones lack β-galactosidase activity. This permits distinction between recombinant and non-recombinant colonies on media with an artificial galactoside that yields a colored product when hydrolyzed.

4. Hessner, et al. specifically show that expression of the *puf* genes is not due to vector sequences but to the endogenous promoter. The *puf* genes were expressed from an endogenous promoter within the 7.7 kb cloned fragment. This 7.7 kb fragment of *R. rubrum* DNA contains the *R. rubrum puf* genes and flanking sequences. Evidence that the *puf* genes were not expressed from vector sequence is listed below:

A. Complementation of the *puf* mutant P5 by the 7.7 kb fragment occurs independently of the orientation of the fragment within the pRK404E1 vector (Table 3, page 5719). If these genes were being expressed from the *lac* promoter of the vector, the expression would be orientation-dependent.

B. Complementation of the *puf* mutant P5 by the 7.7 kb fragment occurs only under anaerobic conditions (page 5720) because the endogenous *puf* promoter is regulated by oxygen. The vector *lac* promoter is not regulated by oxygen and is functional under aerobic conditions.

5. The Examiner further states that "the encoded protein of the vector is considered to be a membrane protein since it complements the deleted *pufB* ..." The *PufB* protein is not encoded by vector sequence but by the cloned fragment within the vector.

6. Hessner, et al. constructed *puf* mutants that are impaired in ICM formation and complemented these mutants with a cloned DNA fragment. Hessner, et al. did not describe these mutants as having excess capacity for membrane formation or describe using them for production of proteins. Hessner, et al. used pRK404E1 as a mobilizable cloning vector not an expression vector. It did not function as an expression vector in this study.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted,

Date: 12/15/04

Mary Lynne Perille-Collins

CURRICULUM VITAE

Mary Lynne Perille Collins
Department of Biological Sciences
University of Wisconsin-Milwaukee
Milwaukee, WI 53201

Present Position: Professor

Academic Training:
B.A. 1971 Emmanuel College, Boston, MA Biology
Ph.D. 1976 Rutgers University, New Brunswick, NJ Microbiology

Awards:
Post-doctoral Fellow, National Institutes of Health NRSA (1976 - 78)
UWM Foundation/Graduate School Research Award (1988)
Graduate School Distinguished Mentor Award (1993)

Professional Experience:
1993-present Professor, Dept. of Biological Sciences, Univ. of Wisconsin-Milwaukee
1986 - 93 Associate Professor, Dept. of Biological Sciences, Univ.of Wisconsin-Milwaukee
1980 - 86 Assistant Professor, Dept. of Biological Sciences, University of Wisconsin-Milwaukee
1978 - 80 Research Assistant Professor, Department of Microbiology, New York University School of Medicine

Selected Publications:

Collins, M. L. P., L. A. Buchholz, and C. C. Remsen. 1991. The effect of copper on *Methylomonas albus* BG8. Appl. Env. Microbiol. 57: 1261-1264.

Hessner, M. H., P. J. Wejksnora, and M. L. P. Collins. 1991. Construction characterization, and complementation of *Rhodospirillum rubrum puf* region mutants. J. Bacteriol. 173: 5712-5722.

Fassell, T. A., L. A. Buchholz, M.L.P. Collins, and C. C. Remsen. 1992. Localization of methanol dehydrogenase in two strains of methylotrophic bacteria detected by immunogold labeling. Appl. Env. Microbiol. 58: 2302-2307.

Lee, I. Y. and M.L.P. Collins. 1993. Identification and partial sequence of the *bch*A gene of *Rhodospirillum rubrum*. Current Microbiol. 27: 85-90.

Buchholz, L.A., J. V. Klump, M. L. P. Collins, C. A. Brantner, and C. C. Remsen. 1995. Activity of methanotrophic bacteria in Green Bay sediments. FEMS Microbiology Ecology 16: 1-8.

Yuan, H., M. L. P. Collins, and W. E. Antholine. 1997. Low-frequency EPR of the copper in particulate methane monooxygenase from *Methylomicrobium albus* BG8. J. Amer. Chem. Soc. 119: 5073-5074.

Brantner, C. A., L. A. Buchholz, C. L. McSwain, L. L. Newcomb, C. C. Remsen, and M. L. P. Collins. 1997. Intracytoplasmic membrane formation in *Methylomicrobium album* BG8 is stimulated by copper in the growth medium. Can. J. Microbiol. 43: 672-676.

Yuan, H., W. E. Antholine, and M. L. P. Collins. 1998. Concentration of Cu, EPR- detectable Cu, and formation of cupric-ferrocyanide in membranes with pMMO. J. Inorganic Biochem. 72: 179-185.

Cheng, Y.S., J. L. Halsey, K. A. Fode, C. C. Remsen, and M. L. P. Collins. 1999. Detection of methanotrophs in groundwater by the PCR. Appl. Environ. Microbiol. 65: 648-651.

Yuan, H., M. L. P. Collins, and W. E. Antholine. 1999. Type 2 Cu^{2+} in pMMO from *Methylomicrobium album* BG8. Biophys. J. 76: 2223-2229.

Brantner, C.A., L. A. Buchholz, C. C. Remsen, and M. L. P. Collins. 2000. Isolation of intracytoplasmic membrane from the methanotrophic bacterium *Methylomicrobium album* BG8. Curr. Microbiol. 40: 132-134.

Cheng, Y. S., C. A. Brantner, A. Tsapin, and M. L. P. Collins. 2000. Role of the H protein in assembly of the photochemical reaction center and intracytoplasmic membrane in *Rhodospirillum rubrum*. J. Bacteriol. 182: 1200-1207.

Lemos, S., M. L. P. Collins, S. S. Eaton, G. R. Eaton, and W. E. Antholine. 2000. Comparison of EPR-visible Cu^{2+} sites in pMMO from *Methylococcus capsulatus* (Bath) and *Methylomicrobium album* BG8. Biophys. J. 79: 1085-1094.

Fode-Vaughan, K. A., C. F. Wimpee, C. C. Remsen and M. L. P. Collins. 2001. Detection of bacteria in environmental samples by Direct PCR without DNA extraction. BioTechniques 31: 598-607.

Brantner, C.A., C. C. Remsen, H. A. Owen, L. A. Buchholz, and M. L. P. Collins. 2002. Intracellular localization of the particulate methane monooxygenase and methanol dehydrogenase in *Methylomicrobium album* BG8. Arch. Microbiol. 178: 59-64.

Lemos, S. S., H. Yuan, M. L. P. Collins, and W. E. Antholine. 2002. Review of multifrequency EPR of copper in particulate methane monooxygenase. Current Topics in Biophysics. Curr. Topics in Biophysics. 26: 43-48.

Fode-Vaughan, K. A.., J. S. Maki, J.A. Benson, and M. L. P. Collins. 2003. Direct detection of *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 37: 239-243.

Benson, J. A., K. A.. Fode-Vaughan, and M. L. P. Collins. 2004. Detection of *Helicobacter pylori* in water by direct PCR. Lett. Appl. Microbiol. 39: 221-225.